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ORIGINAL INVESTIGATION

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Phenotypic assessment of galanin overexpressing and galanin receptor R1 knockout mice in the tail suspension test for depression-related behavior

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Abstract *Rationale:* Galanin and its receptors exert inhibitory neuromodulatory control over brain monoamines. Rat studies revealed that galanin expression is upregulated by exposure to stressors and that galanin manipulations modify neuroendocrine and behavioral responses to stress, leading to the hypothesis that galanin mediates depression-related behaviors. *Methods:* In the present study, we examined the role of galanin in modulating antidepressant-related behavior in galanin overexpressing transgenic (GAL-tg) mice and galanin receptor R1 knockout (GAL-R1 KO) mice, using the tail suspension test (TST). Quantitative autoradiography for 5-HT_{1A}-R and serotonin transporter binding density tested for changes in these two major regulatory components of the 5-HT system in galanin mutant mice. *Results:* Baseline TST behavior was normal in GAL-tg and GAL-R1 KO mice, and intracerebroventricular administration of galanin failed to alter TST behavior in normal C57BL/6J mice. The TST anti-immobility effects of acute treatment with the serotonin reuptake inhibitor, fluoxetine (0–30 mg/kg), and the norepinephrine reuptake inhibitor, desipramine (0–30 mg/kg), were unaltered in galanin mutant mice. Hippocampal 5-HT_{1A}-R density was significantly elevated in GAL-tg and GAL-R1 KO mice, while hippocampal 5-HTT density was reduced in GAL-R1 KO mice, relative to controls. *Conclusion:* Neither pharmacological nor molecular genetic manipulations of galanin altered depression-related profiles in the TST. Possible functional alterations in hippocampal 5-HT neurotransmission may have contributed to these negative results. These preliminary findings provide evidence against the hypothesis that galanin plays a central role in mouse depression-related behaviors. It remains possible that galanin modulates depression-related responses in other experimental paradigms and species.

Keywords Galanin · Antidepressant · Stress · Depression · Mouse · Knockout · Autoradiography · Serotonin · Norepinephrine · Behavior

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Introduction

Galanin is a neuropeptide transmitter implicated in the regulation of diverse behavioral functions, including cognition, nociception, feeding, seizures, sexual behavior, and emotion (Hökfelt et al. 1998a,b; Wrenn and Crawley 2001). These behavioral effects have been linked in part to the ability of galanin to potently inhibit the firing and release of various classical neurotransmitter systems, including norepinephrine (NE), serotonin (5-HT), acetylcholine, and glutamate (e.g. Pieribone et al. 1995; Fuxe et al. 1998; Xu et al. 1998a,b; Kehr et al. 2001, 2002; Ma et al. 2001; Yoshitake et al. 2003, 2004). Moreover, galanin exhibits a marked anatomical coexistence with two major brain monoamines, NE and 5-HT. Galanin is localized in ~80–90% of rat noradrenergic locus coeruleus (LC) neurons, and in ~30% of rat serotonergic dorsal raphe neurons (DRN) (Holets et al. 1988; Hökfelt et al. 1998a,b). In the mouse, a similarly dense galanin coexistence is evident in LC but not DRN neurons (Cheung et al. 2001; Perez et al. 2001; Hohmann et al. 2003; Larm et al. 2003).

The actions of galanin are mediated through at least three G protein-coupled galanin receptors (GAL-R1, GAL-R2, and GAL-R3). Galanin receptors are localized on midbrain monoamine-producing nuclei, as well as their forebrain projection sites, including the amygdala, hippocampus, septum, and hypothalamus (Branchek et al. 2000; Hohmann et al. 2003; Larm et al. 2003).

The anatomical and neuromodulatory characteristics of galanin suggest that this neuropeptide may play an important role in stress. In support of this hypothesis, previous studies have shown that galanin modulates the behavioral, neuroendocrine and sympathetic responses to stressors. Prepro-galanin gene expression in the rat amygdala, hypothalamus and LC is increased by chronic exposure to social, exercise and restraint stress (see Holmes et al. 2003b). Rodent anxiety-related responses are also altered by galanin, albeit in a brain region-specific and task-specific manner. For example, galanin has been found to either reduce or increase anxiety-like behavior in the rat Vogel conflict test when administered into the lateral ventricle or amygdala, respectively (Bing et al. 1993; Moller et al. 1999). In rats treated with the α_2 adrenoreceptor antagonist, yohimbine, and subjected to restraint stress, galanin antagonists microinjected into the amygdala and bed nucleus of the stria terminalis (BNST) exert anxiogenic-like and anxiolytic-like effects on the elevated plus-maze, respectively (Khoshbouei et al. 2002). Recent findings demonstrate that the ability of galanin to attenuate elevated noradrenergic activity extends to a significant attenuation of the physical and behavioral signs of opiate withdrawal in mice (Zachariou et al. 2003).

Taken together, the available research suggests that by inhibiting monoamine neurotransmission, heightened galaninergic activity could exert pro-depressive effects while, conversely, blockade of monoamine-inhibiting effects of galanin may be antidepressant-like (Fuxe et al. 1998; Hökfelt et al. 1998a,b; Weiss et al. 1998; Bellido et al. 2002; Kehr et al. 2002; Holmes et al. 2003a; Zachariou et al. 2003). In support of this hypothesis, Weiss et al. (1998) have shown that bilateral infusion of galanin into the ventral tegmental area, but not the lateral ventricles, midbrain reticular formation or hypothalamus, significantly increased immobility in the rat forced swim test (FST), whereas the galanin antagonist galantide produced an antidepressant reduction in immobility. More recently, Bartfai and colleagues have demonstrated antidepressant-like effects of a novel galanin receptor agonist, galmic in the rat FST (Bartfai et al. 2004). Furthermore, Yoshitake et al. (2003) have recently shown that ICV administration of galanin can significantly attenuate the ability of antidepressants to induce release of 5-HT and NE of in the rat hippocampus. Lastly, [^{125}I]-labeled porcine galanin binding has been found to be increased in the DRN in rats selected for abnormally high depression-like behavior (Bellido et al. 2002).

Galanin overexpressing transgenic (GAL-tg) mice have a conditional overexpression of galanin in epinephrine/NE cells via coupling of the mouse galanin gene to a human dopamine β-hydroxylase promoter (Steiner et al. 2001). The GAL-tg mouse provides a model system for endogenous galanin overexpression (Crawley et al. 2002). Identifying which of the three galanin receptor subtypes may mediate the putative depression-related effects of galanin and galanin overexpression is limited by the current paucity of highly selective non-peptide galanin receptor subtype agonists and antagonists. We took advantage of a recently generated GAL-R1 knockout mouse (Jacoby et al. 2002) to explore the role of the GAL-R1 in depression-related behaviors. Previous studies have found that GAL-tg and GAL-R1 KO mice exhibit cognitive and nociceptive abnormalities, but are normal on a range of motoric, neurological and sensory measures (e.g. Steiner et al. 2001; Wrenn et al. 2004). Pertinent to the present investigation, our previous studies have shown both GAL-tg and GAL-R1 KO mice show evidence of altered anxiety-related behaviors following exposure to stressful test situations or noradrenergic challenges (Holmes et al. 2002a, 2003b).

In the present study, we utilized the GAL-tg and GAL-R1 KO mouse models, in combination with pharmacological approaches, to assess the role of endogenous and exogenous galanin on depression-related behaviors. Depression-related behaviors were assayed using the tail suspension test (TST), a task with predictive validity for measuring the effects of antidepressants in mice (Holmes et al. 2002b; Cryan and Mombereau 2004). A first set of experiments evaluated baseline TST phenotypes in GAL-tg and GAL-R1 KO mice. In a second experiment, the effects of exogenous administration of galanin on TST performance in non-mutant C57BL/6J mice were tested. Thirdly, we evaluated whether TST responses to antidepressants targeting either the 5-HT (fluoxetine) or NE (desipramine) systems were altered in GAL-tg and GAL-R1 KO mice. Growing evidence implicating hippocampal 5-HT neurotransmission in depression-related behavior (Gross et al. 2002; Holmes et al. 2003b), together with the inhibitory effects of galanin on hippocampal 5-HT noted above, suggest depression-related effects of galanin may be mediated at the level of a modulatory influence on the 5-HT system (Fuxe et al. 1998). Therefore, in a fourth set of experiments we performed quantitative autoradiographic analyses of brain binding density of two major components of the 5-HT system, the serotonin transporter and the 5-HT$_{1A}$ receptor.

Materials and methods

Subjects

GAL-tg mice were generated as previously described (Steiner et al. 2001). 129 Sv embryonic stem cells were microinjected into C57BL/6J blastocysts and backcrossed into C57BL/6J for nine generations to avoid complications of a heterogeneous genetic background (Crawley et al. 1997). In order to conditionally overexpress galanin in an anatomically selective manner, the mouse galanin gene was coupled to a human dopamine β-hydroxylase pro-

moter, thereby selectively driving expression in adrenergic cells. GAL-tg mice exhibit a ~5-fold increase in galanin mRNA in the LC and a ~2-fold increase in galanin peptide levels in forebrain projection areas as compared to wild type littermate controls (WT) (Steiner et al. 2001). Galanin peptide levels are approximately fourfold higher in the hippocampus and 10-fold higher in the frontal cortex of GAL-tg mice compared to WT littermates (Wrenn et al. 2002). GAL-R1 receptor knockout (KO) mice were generated as previously described (Jacoby et al. 2002). 129 Sv embryonic stem cells were microinjected into C57BL/6J blastocysts and backcrossed into C57BL/6J for 8–10 generations. RT-PCR analysis of GAL-R1 receptor mRNA levels in the brain and periphery has confirmed that the normal, full-length transcript encoding the GAL-R1 receptor is absent in GAL-R1 KO mice (Jacoby et al. 2002). GAL-tg and GAL-R1 KO mice are viable, develop and reproduce normally. Original founder lines of GAL-tg (University of Washington, Seattle, Wash. USA) and GAL-R1 KO mice (Garvan Institute of Medical Research, Sydney, Australia) were rederived at The Jackson Laboratory (Bar Harbor, Maine, USA) and transported post-weaning to NIMH (Bethesda, Md., USA) at ~8 weeks of age. Twenty-four male C57BL/6J mice were obtained from The Jackson Laboratory at ~8 weeks of age to assess the effects of exogenous galanin. Following receipt at NIMH, all mice were allowed an acclimation period of at least 3 weeks before experiments began. Mice were group housed, two to five per cage, with same-sex littermates, in a temperature-controlled and humidity-controlled vivarium, under a 12 h light/dark cycle (lights on 0600 hours). All experimental procedures were approved by the National Institute of Mental Health Animal Care and Use Committee, and followed the NIH guidelines "Using Animals in Intramural Research."

Behavioral studies in GAL-tg mice were performed using a cohort of 21 male and 22 female GAL-tg mice, and 18 male and 12 female WT littermates. A separate cohort of 6 female GAL-tg mice and 6 of their female WT littermates was used for autoradiographic analysis. Behavioral studies in GAL-R1 mice were performed in three separately bred cohorts. One cohort of 12 male and 12 female KO (KO), 14 male and 12 female heterozygous (HZ), and 14 male and 8 female WT (WT) littermate controls was assessed for baseline TST behavior, and subsequently a random subset of 9 female KO, 6 female HZ and 5 female WT littermate controls was used for autoradiographic analysis. A second cohort of 20 male and 20 female KO, 20 male and 22 female HZ, and 24 male and 18 female WT littermate controls was assessed for the effects of fluoxetine in the TST. A third cohort of 14 male and 15 female KO, 16 male and 16 female HZ, and 20 male and 12 female WT littermate controls was assessed for the effects of desipramine in the TST.

Tail suspension test

The TST for antidepressant activity was conducted as previously described (Holmes et al. 2002a,b; Cryan and Mombereau 2004). Mice were securely fastened with medical adhesive tape by the tip (~1.0–1.5 cm) of the tail to a flat metallic surface and suspended ~30 cm above the ground in a 40 cm^3 white Plexiglas box that isolated the mouse from visual distractions while permitting observation of behavior from above. The presence or absence of immobility, defined as the absence of limb movement, was sampled every 5 s over a 6-min test session by a highly experienced observer who was blind to genotype. An identical procedure was employed for drug challenge experiments.

Cannula implantation and histology

Mice were anesthetized with isoflurane (5% for induction, 2.5% for maintenance) (Baxter Healthcare Corp., Deerfield, Ill., USA) and mounted in a stereotaxic frame (Cartesian Research, Inc., Sandy, Ore., USA). The skull was exposed using a midline incision and a small hole was drilled for cannula placement. For left lateral ventricle placement, the co-ordinates were −0.2 mm posterior, 1.0 mm lateral and 2.5 mm ventral, relative to bregma. A 31-gauge steel guide cannula (Plastics One, Inc., Roanoke, Va., USA) was inserted and secured with a layer of Slow Jet adhesive (Carl Goldberg Models, Inc., Chicago, Ill., USA) and then with dental acrylic (Stoelting, Wood Dale, Ill., USA). In order to keep the cannula patent, a 33-gauge dummy cannula was inserted into the guide cannula. Mice were allowed to recover from surgery for 10 days prior to the start of behavioral testing. At the completion of testing, mice were killed by rapid cervical dislocation, and the brains immediately removed and placed in a 3% formaldehyde solution. Cannula track placement was verified in 50 μ coronal sections stained with thionin (data not shown). Data were removed from analysis for 18 animals in which the cannula did not terminate within the left lateral ventricle.

Quantitative autoradiography of 5-HT_{1A}-R and 5-HTT binding

Quantitative autoradiography of 5-HT_{1A}-R and 5-HTT binding density was conducted as previously described (Holmes et al. 2003c). One month after the completion of GAL-R1 KO behavioral testing (GAL-tg mice were test naive), mice were sacrificed by rapid cervical dislocation. Brains were removed and frozen immediately in dry, ice-cold isopentine for 10 s, transferred to dry ice for 10 min until completely frozen, and then stored at ~80°C. Brains were cut into 16 μm thick coronal sections in a cryostat. The sections were thaw-mounted onto chromalum/gelatin-coated glass slides and stored at ~80°C. To limit variation, each slide contained brain sections from one mouse from

each genotype. Four levels of sections were collected: striatum (bregma 0.98–0.50 mm), medial hypothalamus (bregma 0.7 mm to ~1.06 mm), caudal hypothalamus (bregma ~1.34 mm to ~1.94 mm), and midbrain (bregma ~4.36 mm to ~4.84 mm) according to a mouse brain atlas (Franklin and Paxinos 1997). Adjacent brain sections were used for 5-HT_{1A}-R binding and 5-HTT binding. For 5-HT_{1A}-R binding, sections were pre-incubated for 30 min in an assay buffer (50 mM Tris–HCl, pH 7.4, containing 200 nM $MgCl_2$) and then incubated with ^{125}I-MPPI (0.14 nM in the assay buffer) for 2 h at room temperature. Non-specific binding was defined in the presence of 10^{-5} M 5-HT. Slides were then washed twice with the assay buffer at 4°C for 15 min and rinsed with cold ddH_2O. For 5-HTT binding, sections were pre-incubated in an assay buffer (10 mM sodium phosphate buffer, pH 7.4) for 30 min and then incubated in 20 pM ^{125}I-RTI-55 in the assay buffer. To block dopamine transporter binding to ^{125}I-RTI-55, incubation occurred in the presence of 200 nM of the dopamine transporter inhibitor, GBR 12935. Non-specific binding was defined in the presence of 0.01 mM paroxetine. Slides were then washed twice in assay buffer at 4°C for 20 min and rinsed with cold ddH_2O.

After being air blow-dried, the slides were exposed to Kodak Biomax MR film. Films containing hippocampus and DRN slices were exposed for 1 day. Films containing all other regions were exposed for 3 days. A set of ^{125}I microscales (Amersham Biosciences, Piscataway, N.J., USA) was exposed with the slides to calibrate the optic density into fmol/mg of tissue equivalent. Following exposure, brain images were digitized and analyzed using AIS image software (Imaging research, Inc., Ont., Canada). The gray scale density readings were calibrated to fmol/mg of tissue equivalent using the ^{125}I microscale. Specific ^{125}I-MPPI or ^{125}I-RTI binding in each brain region was determined by subtracting the nonspecific binding sites from the total binding sites in each region. Data for each individual subject and brain region are the mean average of four adjacent sections.

Drugs and chemicals

Fluoxetine hydrochloride, desipramine hydrochloride, 5-HT, and GBR 12935 were obtained from Sigma-RBI (Natick, Mass., USA). Paroxetine was a gift from GlaxoSmithkline. Fluoxetine hydrochloride and desipramine hydrochloride were dissolved in a distilled water vehicle. Injections were given intraperitoneally in a volume of 10 ml/kg body weight 30 min prior to testing. Doses of 10, 20 and 30 mg/kg for both drugs were chosen on the basis of our previous studies of the anti-immobility effects of fluoxetine and desipramine in mice (Holmes et al. 2002b). Galanin (derived from rat, molecular weight, 3164.5) (American Peptide, Inc., Sunnyvale, Calif., USA) was dissolved in sterile distilled water and injected into the left lateral ventricle 5 min prior to testing. Galanin doses of 0.5 nmol (i.e. 1.6 μg) and 1.0 nmol (i.e. 3.2 μg) were chosen on the basis of pilot studies previously conducted in our laboratory. ^{125}I-MPPI and ^{125}I-RTI-55 were obtained from Perkin Elmer (Boston, Mass., USA).

Statistical analysis

There were no significant gender×genotype or gender × drug dose interaction effects on immobility scores in any experiment ($P>0.34$); therefore data were collapsed across gender. For baseline TST experiments and autoradiographic analyses, data were analyzed using either unpaired *t*-test (GAL-tg experiments) or one-factor ANOVA (ICV galanin and GAL-R1 KO experiments). For drug challenge experiments, TST immobility scores were analyzed using a two-factor (genotype×drug treatment) ANOVA, and Newman–Keuls post hoc comparisons. All data were analyzed using StatView (SAS Institute, Inc., Cary, N.C., USA). Statistical significance was set at $P\leq0.05$.

Results

Baseline TST behavior

There was no significant effect of genotype on baseline immobility in the TST in GAL-tg mice (Fig. 1a). Similarly, there was no significant effect of genotype on baseline immobility in the TST in GAL-R1 KO mice (Fig. 1b). Further, there was no significant effect of unilateral ICV galanin administration on immobility in the TST in normal male C57BL/6J mice (Fig. 1c).

Effects of fluoxetine in the TST

GAL-tg mice. There was a significant main effect of drug [$F(3,77)=54.49$, $P<0.001$], but not genotype and no significant drug treatment×genotype interaction, on TST immobility. As shown in Fig. 2a, Newman–Keuls post hoc analysis indicated that fluoxetine significantly reduced immobility in GAL-tg mice at all doses and in WT controls at all doses except 10 mg/kg.

GAL-R1 KO mice. There was a significant main effect of drug treatment [$F(3,112)=30.87$, $P<0.001$], but not genotype and no significant drug treatment×genotype interaction, on TST immobility. As shown in Fig. 2b, Newman–Keuls post hoc analysis indicated that fluoxetine significantly reduced immobility in GAL-R1 KO mice and WT controls at all doses, and in GAL-R1 HZ mice at all doses except 10 mg/kg.

Effects of desipramine in the TST

GAL-tg mice. There was a significant main effect of drug [$F(1,79)=9.42$, $P<0.001$], but not genotype and no significant drug treatment×genotype interaction, on TST immobility. As shown in Fig. 3a, Newman–Keuls post hoc

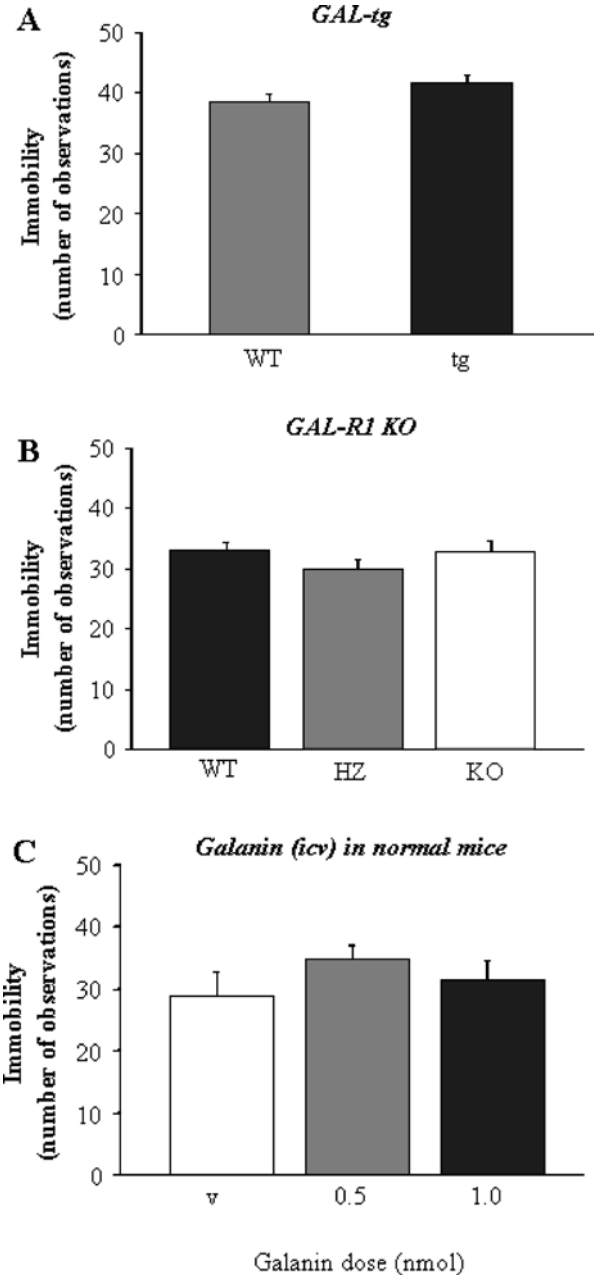

Fig. 1 Neither endogenous galanin overexpression, GAL-R1 knockout nor exogenous galanin administration altered behavior in the tail suspension test (*TST*) for antidepressant activity. There were no differences in immobility **a** between GAL-tg and their wild type (*WT*) littermates, **b** between GAL-R1 KO or HZ mice and their WT littermates, or **c** following unilateral intracerebroventricular administration of galanin in normal C57BL/6J mice. n=7 vehicle; n=10 0.5 nmol; n=7 1.0 nmol. Data in this figure and in Figs 2, 3 are means±SEM. n=41 GAL-tg and n=43 WT littermates; n=24 KO, n=26 HZ, n=22 WT

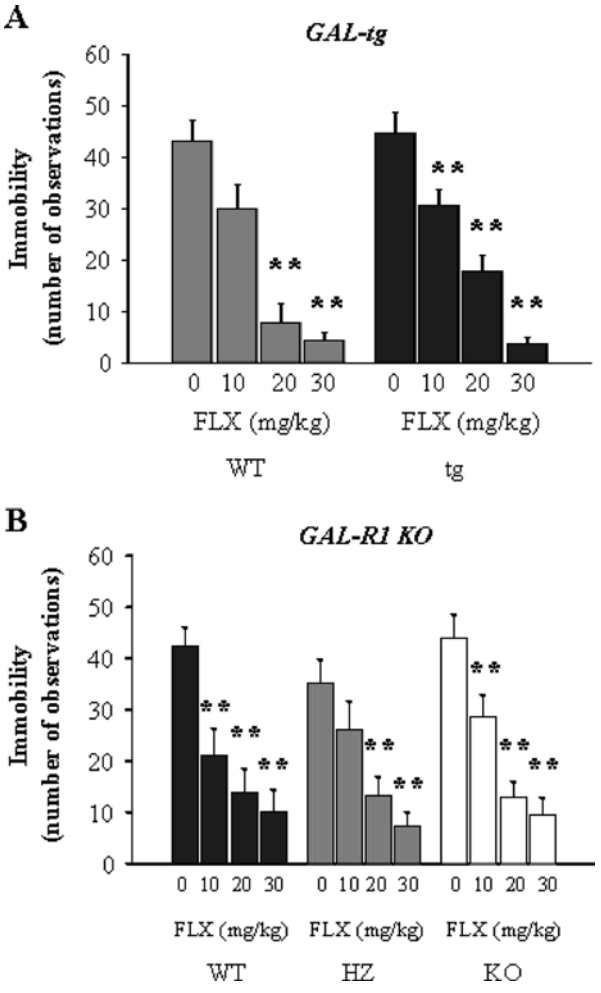

Fig. 2 Galanin overexpressing (*GAL-tg*) mice and GAL-R1 knockout (*KO*) mice showed normal anti-immobility responses to acute treatment with the serotonin reuptake inhibitor, fluoxetine (*FLX*) in the TST. **a** FLX significantly reduced TST immobility in GAL-tg mice at all doses tested, and in their wild type (*WT*) littermates at all doses except 10 mg/kg. **b** FLX significantly reduced TST immobility in GAL-R1 KO and WT mice at all doses tested, and at all doses except 10 mg/kg in GAL-R1 HZ mice. n=10–11 GAL-tg, n=11 WT, per dose. n=9–10 KO, n=10–11 HZ, n=10–11 WT, per dose. **P<0.01 mg/kg versus 0 mg/kg in same genotype

analysis indicated that desipramine significantly reduced immobility in GAL-tg mice at all doses and in WT controls at all doses.

GAL-R1 KO mice. There was a significant main effect of drug treatment [F(3,82)=27.60, P<0.001], but not genotype and no significant drug treatment×genotype interaction, on TST immobility. As shown in Fig. 3b, Newman–Keuls post hoc analysis indicated that desipramine significantly reduced immobility in GAL-R1 KO mice and WT controls at all doses, and in GAL-R1 HZ mice at all doses except 10 mg/kg.

Quantitative autoradiography of 5-HT$_{1A}$-R and 5-HTT binding

Figure 4 shows representative autoradiographs depicting the definition of regions for quantitative autoradiographic analysis. Table 1 summarizes binding data for the 5-HT$_{1A}$-R.

GAL-tg mice. There was significantly greater 5-HT$_{1A}$-R binding density in GAL-tg mice than WT littermates in the CA3 subregion of the hippocampus (t=2.20, df=5, P=0.05), but not other brain regions examined.

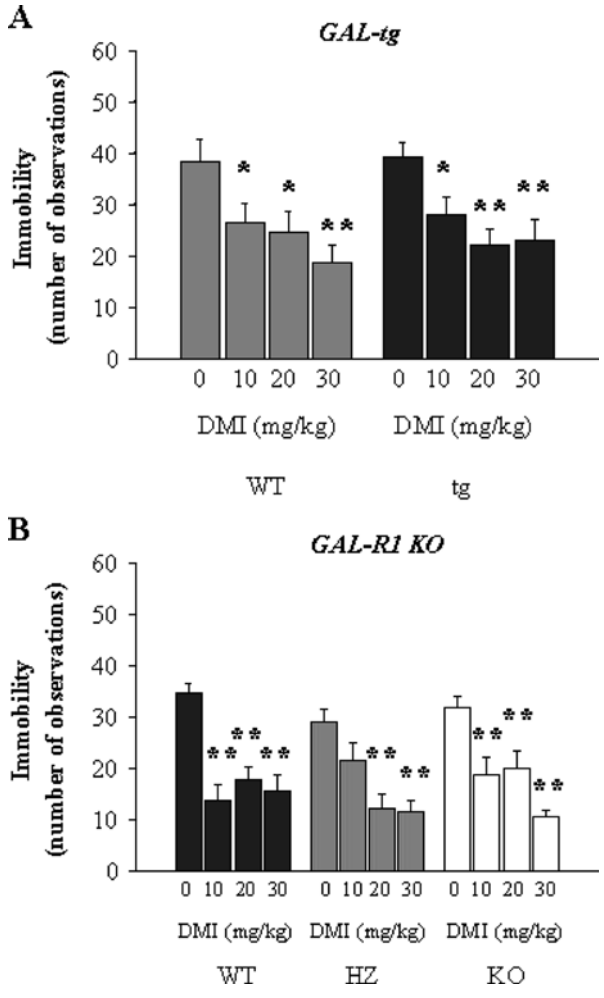

Fig. 3 Galanin overexpressing (*GAL-tg*) mice and GAL-R1 knockout (*KO*) mice showed normal anti-immobility responses to acute treatment with the NE reuptake inhibitor, desipramine (*DMI*). **a** DMI significantly reduced TST immobility in GAL-tg mice and their wild type (*WT*) littermates at all doses tested. DMI significantly reduced TST immobility in GAL-R1 KO, HZ and WT mice at all doses tested. n=10–11 GAL-tg, n=11 WT, per dose. n=7–8 KO, n=7–9 HZ, n=8 WT, per dose. *P<0.05; **P<0.01 vs 0 mg/kg in same genotype

GAL-R1 KO mice. There was a significant effect of genotype on 5-HT_{1A}-R binding density in CA1 [$F(2,14)$ =4.28, P<0.05], CA3 [$F(2,14)$=3.61, P=0.05] and dentate gyrus [$F(2,14)$=5.69, P<0.05], but not other brain regions examined. Newman–Keuls post doc tests indicated that 5-HT_{1A}-R binding was significantly greater in GAL-R1 KO mice than WT littermates in all three of these regions, and in GAL-R1 HZ mice in the dentate gyrus.

Table 2 summarizes binding data for the 5-HTT. *GAL-tg mice*. There were no significant differences in 5-HTT binding density between GAL-tg mice than WT littermates in any of the brain regions examined. *GAL-R1 KO mice*. There was a significant effect of genotype on 5-HTT binding density in CA3 [$F(2,14)$=4.45, P<0.05] and lateral septal nucleus [$F(2,14)$=6.99, P<0.01], but not other brain regions examined. Newman–Keuls post doc tests indicated that 5-HTT binding was significantly greater in GAL-R1 KO and HZ mice than WT littermates in the CA3 and lateral septal nucleus.

Table 1 Quantitative autoradiographic analysis of 5-HT_{1A}-R binding density in galanin overexpressing (*GAL-tg*) mice and GAL-R1 knockout (*KO*) mice. There was a significantly higher density of ^{125}-I-MPPI-labeled 5-HT_{1A}-R binding sites in GAL-tg mice than their wild type (*WT*) littermates in the CA3 subregion of the hippocampus, but not in other brain regions. There was a significantly higher density of ^{125}-I-MPPI-labeled 5-HT_{1A}-R binding sites in GAL-R1 KO mice than WT littermates in the CA1, CA3 and dentate gyrus regions of the hippocampus, but not in other brain regions. GAL-R1 HZ mice also showed significantly higher density of ^{125}-I-MPPI-labeled 5-HT_{1A}-R binding sites than WT littermates in the CA3 region. Data are means ± SEM expressed in fmol/mg tissue equivalent. n=5–6 per genotype. *P≤0.05, **P<0.01 versus corresponding WT littermate controls. *AC* central amygdaloid nucleus; *AH* anterior hypothalamic nucleus; *BLA* basolateral amygdaloid nucleus, anterior; *BMA* basomedial amygdaloid; *CA1* CA1 subfield of hippocampus; *CA3* CA3 subfield of hippocampus; *DG* dentate gyrus; *DMN* dorsomedial hypothalamic nucleus; *DR* dorsal raphe nucleus; *Ld* lambdoid septal zone; *LHN* lateral hypothalamic nucleus; *LSI* lateral septal nucleus, intermediate; *MR* medial raphe nucleus; *MS* medial septal nucleus; *PVN* paraventricular hypothalamic nucleus; *VMN* ventromedial hypothalamic nucleus

	GAL-tg		GAL-R1 KO		
	WT	GAL-tg	WT	HZ	KO
Hippocampus					
CA1	38.7±1.9	39.6±1.6	36.1±3.1	42.2±2.4	45.7±1.4*
CA3	5.0±0.2	6.1±0.4*	4.8±0.7	6.0 ±0.3	6.7±0.5*
DG	5.7±0.5	5.8±0.6	5.0±0.6	7.0±0.3*	7.5±0.6*
Amygdala					
AC	8.2±0.8	8.5±0.9	8.1± 0.9	8.5±0.5	9.2±0.7
BLA	5.6±0.3	5.4±0.4	4.7±0.5	6.0±0.6	5.4±0.5
BMA	8.7±0.4	9.1±0.4	9.7±0.5	8.6±0.5	9.7±0.9
Septum					
Ld	25.1±0.8	24.8±0.6	25.0±1.0	24.1±1.0	24.1±0.6
LSI	18.0±0.6	16.5±0.5	17.4±1.0	18.2±0.9	17.7±0.7
MS	26.9±0.6	26.5±2.0	26.2±3.3	25.4±1.4	24.6±1.3
Hypothalamus					
AH	9.0±0.3	9.6±0.5	7.0±0.3	6.8±0.3	8.0±0.4
DMH	5.8±0.3	5.5±0.4	6.2±0.4	6.1±0.3	6.7±0.5
LHN	3.4±0.2	3.9±0.4	3.0±0.2	2.7±0.2	2.9±0.2
PVN	8.1±0.8	7.6±1.0	3.0±0.3	3.0±0.4	3.7±0.3
VMH	3.9±0.4	4.5±0.5	5.6±0.5	6.1±0.7	6.6±0.9
Midbrain					
DR	38.9±2.3	39.4±2.7	39.2±3.2	37.2±2.1	38.1±1.5
MR	13.6±1.2	14.6±1.4	13.0±0.5	12.5±0.5	13.2±0.5

Discussion

Galaninergic modulation of depression-related behavior and responsivity to antidepressants was assessed in the mouse TST using a combination of pharmacological, neurochemical and gene mutant approaches. Transgenic mice with a conditional overexpression of galanin in adrenergic neurons (GAL-tg) showed TST scores that were no different from WT littermates. Similarly, mice with a homozygous targeted null mutation of the galanin

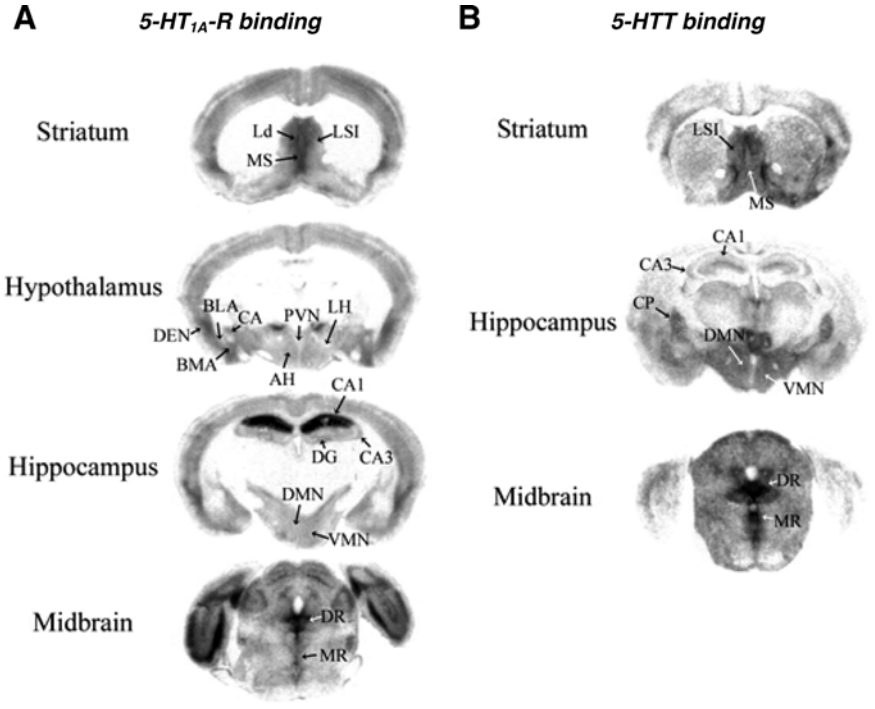

Fig. 4 Representative autoradiographs from wild type mice depicting the definition of regions for quantitative analysis of **a** ^{125}I-MPPI, 5-HT$_{1A}$-R binding and **b** ^{125}I-RTI-55, 5-HTT binding. See Table 1 for key to abbreviations

Table 2 Quantitative autoradiographic analysis of 5-HTT binding density in galanin overexpressing (*GAL-tg*) mice and GAL-R1 knockout (*KO*) mice. There were no differences in the density of ^{125}-I-RTI-labeled 5-HTT binding sites between GAL-tg mice and wild type (*WT*) littermates in any brain region examined. There was a significantly lower density of ^{125}-I-RTI-labeled 5-HTT binding sites in GAL-R1 KO and GAL-R1 HZ mice than WT littermates in the CA3 region of the hippocampus and the intermediate division of the lateral septal nucleus, but not other brain regions. Data are means ±SEM expressed in fmol/mg tissue equivalent. n=5–6 per genotype. *$P \leq 0.05$ versus corresponding WT littermate controls. *CP* caudate putamen, see Table 1 for key to other abbreviations

	GAL-tg		GAL-R1 KO		
	WT	GAL-tg	WT	HZ	KO
Hippocampus					
CA1	3.9±0.4	4.4±0.8	4.9±0.8	4.7±0.4	4.7±0.2
CA3	4.1±0.5	4.1±0.3	5.7±0.2	4.5±0.3*	4.7±0.3*
Septum					
LSI	8.5±0.5	9.4±0.7	9.9±0.4	8.3±0.3**	8.5±0.4**
MS	9.0±0.4	9.2±0.3	9.2±0.9	8.9±0.4	9.0±0.5
Hypothalamus					
DHM	13.1±0.9	14.0±0.4	11.7±0.9	12.7±1.3	12.4±0.7
VMH	9.7±0.9	9.4±0.8	8.2±1.3	7.4±0.8	8.1±0.5
Midbrain					
DR	17.8±1.1	18.1±1.4	17.9±0.9	18.2±0.6	16.0±1.2
MR	10.7±0.6	10.9±1.0	10.4±0.7	11.1±0.5	10.8±0.5
Striatum	5.3±0.4	5.3±0.3	5.0±0.2	5.2±0.2	5.0±0.2
CP	9.0±0.4	9.6±0.4	5.9±0.7	6.7±0.6	6.4±0.7

receptor R1 subtype (GAL-R1 KO) were similar to heterozygous and WT littermates on the TST. It is unlikely that these normal phenotypes were complicated by gross behavioral or neurological abnormalities (see Steiner et al. 2001; Wrenn et al. 2004). Moreover, providing pharmacological support for these findings, ICV administration of galanin in non-mutant C57BL/6J mice did not alter behavior in this test. Thus, the conclusion of these experiments is that neither endogenous nor exogenous manipulations of galanin alter baseline behavior in the mouse TST.

Responses to fluoxetine, a 5-HT reuptake inhibitor and desipramine, a NE reuptake inhibitor, were evaluated in galanin mutant mice. As expected, acute treatment with either fluoxetine or desipramine produced significant reductions in TST immobility in WT mice. Extending the normal baseline behavioral phenotypes in GAL-tg and GAL-R1 KO mice, the antidepressant-related effects of both drugs were normal in these mutants, demonstrating that neither galanin overexpression nor GAL-R1 inactivation modified antidepressant-mediated behavioral effects in the TST.

The absence of altered depression-related behaviors or antidepressant-responsivity in galanin mutant mice does not concur with a previous report demonstrating significantly increased immobility in the rat FST following microinjection of galanin into the ventral tegmental area (Weiss et al. 1998), or with a recent study showing that a systemic administration of a non-peptide galanin agonist exerts apparent antidepressant-like activity in the same test (Bartfai et al. 2004). There is also strong evidence of interactions between galanin and the monoamine systems that mediate stress. Electrophysiological studies in rats have consistently demonstrated inhibitory effects of galanin on NE and 5-HT neuronal firing (Pieribone et al. 1995; Xu et al. 1998a,b; Ma et al. 2001). In vivo microdialysis studies in both rats and mice further indicate potent inhibitory effects of ICV galanin on basal and stress-induced 5-HT and NE release in the hippocampus

(Kehr et al. 2001, 2002; Yoshitake et al. 2003, 2004). In addition, and of particular note in the context of present findings, ICV galanin has recently been shown to significantly inhibit hippocampal 5-HT and NE release induced by acute antidepressant treatment in rats (Yoshitake et al. 2003) at doses comparable to those used in our study. In this context, the absence of altered behavioral responses to antidepressants in galanin mutant mice is somewhat unexpected.

Neurophysiological evidence suggests that neuropeptides such as galanin are preferentially released under conditions of high neuronal activity, such as those likely to occur in response to extreme or prolonged stress (Hökfelt et al. 1987; Consolo et al. 1994). We have previously found that GAL-tg and GAL-R1 KO mice display abnormal anxiety-like phenotypes only in response to a NE-acting anxiogenic (yohimbine) or under conditions of relatively high stress (Holmes et al. 2002a, 2003b). These observations are consistent with rat studies showing that anxiety-related effects of galanin treatment are often restricted to relatively stressful and hyper-noradrenergic states and that galanin mRNA is upregulated primarily by strong stressors such as social defeat and chronic restraint, but not by less intense manipulations such as wheel running (see Khoshbouei et al. 2002; Holmes et al. 2003b). On the basis of these findings, it will be of interest to assess whether chronic stress is necessary to recruit galaninergic modulation of depression-related behavior, using galanin mutant mice and other approaches. Moreover, because monoamine activity is expected to be higher following chronic than acute antidepressant treatment (Blier and Ward 2003), it is possible that the effects of galaninergic inhibition of monoamine function would be more pronounced following chronic treatment regimes.

The potential for compensatory mechanisms that mask the effects of lifetime gene mutations is widely recognized (Crawley 2000). An alternative explanation of the present negative findings in GAL-tg and GAL-R1 KO mice is that developmental compensatory alterations in monoamine systems may have masked the modulatory effects of galanin on TST behavior. The capacity for such changes has been underscored in recent investigations. Thus, GAL-tg mice exposed to another rodent test for antidepressant-related activity, the FST, showed an unexpected exaggeration of hippocampal NE and 5-HT release, an effect strikingly at odds with the normally inhibitory effects of galanin treatment in normal mice (Yoshitake et al. 2004). We found that galanin mutations cause alterations in 5-HT_{1A} receptor subtype and 5-HT transporter (5-HTT) binding. Autoradiographic analysis demonstrated increased 5-HT_{1A}-R binding in CA1, CA3 and dentate gyrus subregions of the GAL-R1 KO hippocampus and in the CA3 of GAL-tg mice, relative to WT controls. In addition, binding density of the 5-HTT was significantly decreased in CA3 and lateral septum in GAL-R1 KO mice. Alterations in 5-HT_{1A}-R or 5-HTT binding were not seen in other forebrain regions, such as the amygdala and hypothalamus, nor in the vicinity of the serotonergic midbrain raphe nuclei, indicating a specific alteration in the hippocampus. These changes could reflect an upregulation of 5-HT function in this region, i.e. increased postsynaptic 5-HT_{1A}-R density, decreased 5-HTT-mediated clearance of 5-HT.

5-HT neurotransmission in regions including the hippocampus is strongly implicated in depression-related and stress-related behavior (Gross et al. 2002; Holmes et al. 2003b), and it is reasonable to propose that galanin's putative depression-related effects may be mediated via modulatory effects on hippocampal 5-HT. In addition to galanin's inhibitory effects on hippocampal 5-HT release, there is growing evidence that galanin functionally antagonizes 5-HT_{1A}-R function. Administration of galanin (ICV) in rats reduces 5-HT_{1A}-R mRNA and protein binding in the DRN (Razani et al. 2000), decreases 5-HT_{1A}-R binding affinity and prevents 5-HT_{1A}-R autoreceptor inhibition of hippocampal 5-HT release (Kehr et al. 2002; Yoshitake et al. 2003). Moreover, behavioral studies in rats have demonstrated that ICV galanin attenuates the cognitive-impairing, hypothermia-inducing and hypoactivity-inducing effects of 5-HT_{1A}-R agonists (e.g. Misane et al. 1998; Kehr et al. 2002). Thus, it remains possible that the presently observed alterations in hippocampal 5-HT_{1A}-R function in galanin mutants may have compensated for abnormal galaninergic modulation of 5-HT function and thereby diminished any depression-related abnormalities. This suggestion will remain speculative until a direct link between galanin and hippocampal 5-HT function in the mediation of depression-related behaviors can be established.

Future studies will also clarify the potential role of the other galanin receptor subtypes in mediating the effects of galanin on stress-related and depression-related behaviors. Based on existing anatomical and electrophysiological data, it has been suggested that the GAL-R1, acting as an autoreceptor, mediates galaninergic inhibition of LC NE activity, whereas the GAL-R2 may govern galanin inhibition of DRN 5-HT activity (Xu et al. 1998b; Branchek et al. 2000; Ma et al. 2001; Hohmann et al. 2003; Zachariou et al. 2003; Yoshitake et al. 2004). In addition, like the GAL-R1, the GAL-R2, and to a much lesser extent GAL-R3, are distributed in limbic forebrain regions mediating emotion and stress (Branchek et al. 2000; Hohmann et al. 2003). Thus the availability of GAL-R2 KO and GAL-R3 KO mice and galanin receptor subtype-specific ligands to further assess galaninergic modulation of depression-related behavior are awaited. Lastly, given recent evidence that in contrast to the high level of galanin-5-HT colocalization in the rat DRN, there is a near-absence of galanin and GAL-R1 expression in mouse DRN (Cheung et al. 2001; Perez et al. 2001; Hohmann et al. 2003; Larm et al. 2003), it will be critical to verify present findings on the role of GAL-R1 in depression-related behavior in other species, particularly rats.

In summary, the present study showed that mice either overexpressing galanin or lacking a functional GAL-R1 performed normally on the TST for depression-related behavior. GAL-tg and GAL-R1 mice also exhibited intact anti-immobility responses to acute treatment with fluox-

etine and desipramine, antidepressants that target the 5-HT and NE systems, respectively. Analysis of 5-HT_{1A}-R and 5-HTT binding densities provided evidence of an alteration in 5-HT function in the hippocampus in galanin mutant mice. Taken together, these initial data argue against a major role for galanin in mediating mouse depression-related behaviors. Further research using alternative tasks, treatment regimes and species is warranted.

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